SUPPRESSION OF NONSENSE BY REVERTANT BACTERIA

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The translation of the genetic code into a polypeptide chain involves recognition by a transfer RNA of a specific three base codon. If this transfer RNA is able to recognize the codon but introduces an abnormal amino acid or no amino acid at all the translation process will become altered or abortive, respectively. Mutant cells have been discovered which presumably possess an altered codon resulting in the interruption of the polypeptide chain (Garen and Siddiqi, 1962). Such altered codons have been termed nonsense codons and the mutations are referred to as nonsense mutations (Benzer and Champe, 1962). The bases have been identified in two nonsense codons and are UAA and UAG (Brenner, Stretton and Kaplan, 1965). A cell which has a transfer RNA that recognizes the nonsense codon in a constructive way will completely translate the genetic message by the insertion of an amino acid at the position in the polypeptide chain coded by the nonsense codon (Weigert and Garen 1965; Stretton and Brenner, 1965; Weigert, Lanka and Garen, 1965; Kaplan, Stretton and Brenner, 1965). Revertant bacteria which do this have been isolated from mutant cells that contain a nonsense codon (Weigert and Garen 1965). In such cells altered codon recognition is thought to be the basis of mutation, indicating that the base sequence has been altered in the stretch of DNA that codes for transfer RNA (Capecchi and Gussin, 1965; Engelhardt, Webster, Wilhelm and Zinder, 1965).

The amber mutants of T4 phage offer a powerful technique for examining whether a mutation is in the class affecting the transfer

RNA. An amber mutant has been used in these studies to examine whether revertant bacteria of E. coli WWU, produced by ultraviolet and ionizing radiations as well as by uracil-5H^3 radioactive decays, are in this class. Our data show that phenotypic reversion in WWU occurs frequently by this mechanism.

MATERIALS AND METHODS

Stocks: T4, T4 amber B22, E. coli CR63 and E. coli B were given us by Dr. William Ginoza of this department. T4 amber B22 was originally obtained from Dr. R. S. Edgar (California Institute of Technology). E. coli WWU, a polyauxotroph, has been described by Person and Bockrath (1964). While T4 plated on all these host cells T4 amber B22 plated only on CR63.

Methods: WWU was grown in minimal medium, filtered and resuspended by the method of Person and Bockrath (1964). After the mutagenic treatment 0.2 ml of undiluted suspension was plated to isolate revertants of the arginine locus. Plating conditions were those of Person and Bockrath (1964) except that arginine at 3.5 μg/ml replaced nutrient broth at 0.2 mg/ml in the plating medium. This concentration of arginine allows maximum mutation expression. Revertant plates were incubated for 36 hours at 37°C. Individual revertants were purified by streaking on similar medium lacking arginine and regrown. An innoculum from each streak was placed in 1-2 ml of nutrient broth (8 g/l) containing thymidine (40mg/l) and shaken at 37°C until the cell number reached 10^9/ml. The cultures were held at 4°C until used as hosts in the suppression test.

Mutagenic treatments: Ultraviolet Light. A germicidal lamp was used to deliver a dose of 300 ergs/mm^2 to 10 ml of culture in a 100mm diameter glass petri dish.

Ionizing Radiation (Co^{60}). Doses given were: liquid suspension (0°C) 4350 rads; liquid suspension containing catalase at 2 µg/ml (0°C) 8700 rads (Catalase from General Biochemicals, Ohio); frozen sample (-78°C) 8700 rads.

Uracil-5H^3 revertants were produced by the method of Person and Bockrath (1964).

Phage assay plates: Bottom agar (1.5%) contained tryptone broth (12 g/1), yeast extract (10 g/1), and sodium chloride (5.8 g/1). Top agar (0.4%) contained nutrient broth (8 g/1) and sodium chloride (5.8 g/1). Thymidine (40 mg/1) was added to top and bottom agar to prevent viability decline, a phenomenon that occurs in some su+ revertants of WWU (Bockrath, 1965).

RESULTS

The results of experiments testing the ability of revertant bacteria to suppress T4 amber B22 are shown in the table. All su- revertants gave approximately 5 plaques per 10^7 phage plated and some of these were tested and found to be T4. Su+ revertants on the other hand had an efficiency of plating of B22 of 1-100% relative to CR 63, depending on the individual revertant and the mutagenic agent used.

SUPPRESSION OF T4 AMBER B22 BY REVERTANTS OF WWU INDUCED BY VARIOUS AGENTS.

Mutagenic agent	No. independent isolations of revertants	Colonies tested(*)	No. su+(*)	% su+
Ionizing radiation				
liquid	4	136	74	55
frozen	3	69	52	76
catalase	3	81	60	74
Ultraviolet light	3	115	95	83
Uracil-$5H^3$ decay	3	136	136	100

* The data has been corrected for revertants of spontaneous orgin appearing on the treated plates. Separate controls were not run for the frozen and catalase cultures.

The percentage of su+ revertants for amber B22 was reproducible from isolation to isolation ($\pm$ 10%) although the frequency of su+ revertants among independent isolates of spontaneous revertants varied between 20% and 60%. We believe this variation in the spontaneously produced revertants to be real and it could reflect the time at which a su+ revertant is formed in the growth tube.

It is clear that the percentage of revertants which can suppress B22 is dependent on the mutagenic agent. For cells irradiated in the liquid state with ionizing radiation 55% of the revertants formed are su+. If cells are irradiated in the frozen state, or in the presence of catalase, to minimize the indirect effect, the percentage of revertants that can suppress amber B22 is increased. 83% of the revertants produced by ultraviolet light are able to suppress B22. Perhaps the most interesting result is that all revertants produced by uracil-$5H^3$ decay are su+ for B22. The high mutagenic effect of uracil-$5H^3$,

which has been shown by Person and Bockrath (1965) to be associated with a chemical change associated with the decay, therefore results in reversion by means of a change outside the structural gene.

DISCUSSION

The finding that some arginine revertants of WWU will suppress a T4 amber mutant whereas the parent WWU will not indicates (1) that the original mutational alteration preventing the formation of arginine is a nonsense codon and (2) that reversion sometimes occurs by an alteration outside the structural gene probably by formation of an sRNA able to suppress the nonsense codon.

Reversion is assumed to be a single event, and it is known from the work of Weigert and Garen (1965) and of Stretton and Brenner (1965) that a su+ revertant inserts a specific amino acid on reading the nonsense codon. In our experiments a revertant giving a su+ result had to insert an amino acid which not only resulted in a phenotypically arginine independent bacterium, but also read the UAG codon so as to make complete functional phage protein. It is clear therefore that these estimates for the per cent suppression are a minimum and that they may vary according to the su- locus examined and also with respect to the amber phage used. However since the percentage of the revertants that can suppress one amber varies according to the mutagenic treatment given it is concluded that the events leading to reversion with ultraviolet light, ionizing radiation and uracil-$5H^3$ decay are not identical.

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